

FROM THE DEPARTMENT OF MOLECULAR MEDICINE  
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**INFLUENCE OF ALCOHOL  
ON SOME APPETITE-  
REGULATING HORMONES  
IN MAN**

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*Noah, a man of the soil, was the first to plant a vineyard.*

*Genesis 9:20*

*Och Noa var en åkerman och var den förste som planterade en vingård*

*Genesis 9:20*



## ABSTRACT

Beverage containing alcohol has been used for centuries to stimulate appetite. Ingestion of a moderate amount of alcohol increase energy intake. Regulation of food intake is complex. Several factors cooperate such as neural impulses from sensory organs; sight, smell, gut distension, social setting, memory, current energy status and hormones. How alcohol affects these factors is not well understood. This research project has focused on how alcohol influences the peripheral secretion of hormones, known to convey information from the periphery to hunger-regulating centers in the brain about the prevailing caloric homeostasis. Leptin – produced by adipocytes -and gut-derived peptide YY (PYY) are two such hormones which inhibit food intake, whereas ghrelin – produced by cells in the upper part of the gastro intestinal tract - stimulates appetite. These hormones have central effects on hypothalamic neuropeptide Y (NPY) and on pro-opiomelanocortin (POMC) which stimulates and down-regulates hunger, respectively. Other gut hormones, having central effects as well as influence on intestinal motility, are glucagon-like peptide (GLP-1) and obestatin. Liver derived insulin-like growth factor-1 (IGF-1) and its binding protein insulin-like growth factor binding protein-1 (IGFBP-1) are also of interest in this context, as they are affected by nutritional status and could be factors which influence appetite-regulating centers directly or indirectly via peripherally produced hormones.

**Aim:** To study the effect of alcohol on the secretion of peripheral hormones known to be involved in the regulation of food intake.

**Material and Methods:** 51 healthy subjects (26F/25M) were included in the study. All were young, healthy, normal weight and free of medication. In five separate experiments subjects were investigated in groups of 7 – 12 individuals. In *exp 1, 2 and 3* the effect of alcohol on various hormone levels in serum, was compared with the effect of drinking water. In *exp 2* the alcohol effect on urinary excretion of catecholamines was determined with or without oral beta-receptor blockade (propranolol). In *exp 5* alcohol influence on gastro-intestinal hormones was investigated with or without sucralfate gastroprotection.

**Results and discussion:** A moderate amount of alcohol induced a significant inhibition of both diurnal and nocturnal secretion of leptin. Factors known to affect the secretion of leptin such as insulin, glucose, cortisol, testosterone, and catecholamines were not influenced by the drug. IGFBP-1 increased significantly after alcohol, contrasting IGF-1, which remained unchanged. This resulted in a low IGF-1/IGFBP-1 ratio and, as a consequence, in a decreased IGF-1 bioavailability. Alcohol had both acute and prolonged inhibitory effect on serum levels of both total and octanoylated ghrelin, but was without significant influence on serum concentrations of NPY, PYY, GLP-1 and obestatin. Gastro-protection with sucralfate did not change the alcohol-induced inhibition of leptin and ghrelin secretion.

**Conclusion:** Acute ingestion of alcohol inhibits the secretion of leptin and ghrelin, induces a marked decline in the IGF-1/IGFBP-1 ratio, but leaves NPY, PYY, GLP-1 and obestatin unchanged. Previous studies suggest that leptin may have long-term rather than acute inhibitory effects on hunger. Therefore, the present findings do not lend strong support to the hypothesis that alcohol has acute stimulatory effect on appetite by influencing peripherally produced hormones. If alcohol has appetite-stimulating properties in humans it is more likely that this is an effect caused by direct influence on appetite-regulating neurons in the brain.

**Key words:** leptin, ghrelin, octanoylated, PYY<sup>3-36</sup>, GLP-1, obestatin, alcohol, NPY, IGF-1, insulin, glucose, testosterone, cortisol, catecholamines



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# 1 LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

- I Röjdmark S, **Calissendorff J**, Brismar K. Alcohol decreases both diurnal and nocturnal secretion of leptin in healthy individuals. *Clinical Endocrinology* 2001; 55:639-647
- II **Calissendorff J**, Brismar K, Röjdmark S. Is decreased leptin secretion after alcohol ingestion cathecholamine-mediated? *Alcohol & Alcoholism* 2004;39:281-286
- III **Calissendorff J**, Danielsson O, Brismar K, Röjdmark S. Inhibitory effect on ghrelin secretion in normal man. *European Journal of Endocrinology* 2005; 152:743-747
- IV **Calissendorff J**, Danielsson O, Brismar K, Röjdmark S. Alcohol ingestion does not affect serum levels of peptide YY but decreases both total and octanoylated ghrelin levels in healthy subjects. *Metabolism* 2006, 55:1625-1629
- V **Calissendorff J**, Gustafsson T, Holst JJ, Brismar K, Röjdmark S. Effect of alcohol on some appetite-regulating hormones in man. Does gastroprotection with sucralfate change the alcohol effect? *submitted*

## RELATED ARTICLES NOT INCLUDED IN THE THESIS

1. Ronis MJJ, Wands JR, Badger TM, de la Monte SM, Lang CH, **Calissendorff J.** Alcohol-induced disruption of endocrine signaling. *Alcohol Clinical and Experimental Research*. 2007, Vol 31, No 8,:1-17

## **2 LIST OF ABBREVIATIONS**

Acetyl-CoA	Acetyl co enzyme A
ALS	Acid labile subunit
AgRP	Agouti related peptide
ADH	Alcohol dehydrogenase
ACTH	Adrenocorticotropic hormone
ARC	Arcuate nucleus
ATP	Adenotriphosphate
BPs	Binding proteins
CART	Cocaine and amphetamine-regulated transcript
CCK	Cholecystokinin
CNS	Central nervous system
CRF	Corticotropin releasing factor
CV	Coefficiency of Variance
DPP-IV	Dipeptidyl peptidase IV
ELISA	Enzyme-linked immunosorbent assay
IGF-1	Insulin-like growth factor-1
IGFBP-1	Insulin-like growth factor binding protein-1
IFN $\alpha$	Interferon alpha
Il	Interleukin
GH	Growth hormone
GHRH	Growth hormone releasing hormone
GLP-1	Glucagon-like peptide-1
GRP	Gastrin related peptide
MCH	Melanin-concentrating hormone
MEOS	Microsomal ethanol oxidizing system
NAD	Nicotanimide adenine dinucleotide
NPY	Neuropeptide Y
NTS	Nucleus Tractus Solitarius
PCR	Polymerase chain reaction
POMC	Pro-opiomelanocortin
PVN	Paraventricular nucleus
PYY <sup>3-36</sup>	Peptide YY
RIA	Radioimmunoassay
TNF- $\alpha$	Tumor necrosis factor alpha

### **3 THESIS SUMMARY – MAIN SECTION**

Alcohol is used by people all over the world, and is probably one of the most frequently used drugs both in history and today. Its appetite-stimulating properties have been recognized for centuries but little scientific evidence has been presented to show how this effect is brought about. Today most investigators in this field believe that hunger and satiety are regulated by a complex interplay between specific centers in the brain and signals from peripheral tissues, bringing information to the brain about energy balance and caloric needs of the body. These peripheral signals are conveyed by hormones, which thus have an important role to play in the regulatory process. It is possible that alcohol, by affecting these hormonal signals, also influences appetite and food intake. This research project focuses on how moderate amounts of alcohol influence the secretion of appetite-regulating hormones in normal subjects.

**Material and methods:** 51 healthy, non-addicted individuals were included in the study (26F/25M). In all experimental settings these participants served as their own controls. The effect of alcohol ingestion on appetite regulating hormones was compared with that of drinking water or placebo. Such experiments were performed during the day as well as during the night, and lasted from 1 to 14 hours. It was also studied whether oral administration of propranolol (a  $\beta$ -adrenoreceptor blocking agent) or sucralfate (a gastroprotective drug) would change the alcohol effect on the hormone secretion.

**Results:** *Leptin is known to decrease hunger.* In this study both diurnal and nocturnal serum levels of leptin decreased significantly after acute ingestion of alcohol.

*IGF-1 appears to inhibit leptin, and may therefore indirectly influence hunger.* In the present investigation alcohol increased the IGFBP-1 level significantly, but left the IGF-1 level unchanged. This resulted in a low IGF-1/IGFBP-1 ratio and, as a

consequence, in a decreased IGF-1 bioavailability. Other factors known to affect the secretion of leptin, such as insulin, glucose, cortisol, testosterone and catecholamines, were not influenced by acute ingestion of alcohol.

*Ghrelin is known to stimulate appetite via vagal afferents to appetite regulating centers within the brain.* In the current investigation alcohol had both acute and prolonged inhibitory effect on the secretion of this hormone. Moreover, it had similar effect on the secretion of both octanoylated and total ghrelin. These hormones are known to be biologically active and inactive, respectively.

Other hormones influencing appetite and hunger via vagal reflexes, such as GLP-1, PYY, and obestatin were not affected by alcohol nor was peripheral NPY.

*Ghrelin is secreted by cells in the gastrointestinal tract. Small amounts of leptin may also be produced there.* In our series gastroprotection with sucralfate did not change the alcohol-induced inhibition of leptin and ghrelin secretion.

**Conclusion:** If alcohol has appetite-stimulating properties, one would expect to find inhibitory effect on leptin secretion and IGF-1 bioavailability. One would also expect increased secretion of ghrelin after ingestion of the drug. Contrary to what was expected ghrelin levels fell markedly in the present investigation. Although leptin levels and IGF-1 bioavailability also decreased significantly, this does not necessarily mean that alcohol-induced leptin changes are the mechanism underlying alcohol-induced appetite stimulation. The reason for this is that alcohol was ingested acutely in the present investigation. Previous studies suggest that leptin may have a long-term rather than acute inhibitory effect on hunger. Therefore, the present findings do not lend strong support to the hypothesis that alcohol has acute stimulatory effect on appetite by influencing peripherally produced hormones. If alcohol has appetite stimulating properties in man it is more likely that this is an effect caused by direct influence on appetite-regulating neurons in the CNS.

## 4 INTRODUCTION

The knowledge of how to produce alcohol-containing beverage is probably more than 5 000 years old, and appetite stimulating properties have on empirical grounds been attributed to wine for almost 2000 years.<sup>1</sup> Already in ancient Greece this was empirically known, believed to be a gift from the wine God, Dionysos.<sup>2</sup> In the ancient Kyrgyz nation mare's milk was fermented and this *kumys* was known to be nourishing and stimulating, improving hunger and work ability.<sup>3</sup> During the Middle Ages, when local wars were common, and tended to be ongoing almost continuously, the distillation technique opened up new ways to treat injured soldiers, and also diseased civilians, as strong alcoholic drinks proved efficient to kill pain and induce tranquility.<sup>4</sup> Later on, during the 19<sup>th</sup> century, the psychoactive sides of the drug came in to focus, when social life flourished, and aperitifs, which refers to appetite, became popular.<sup>5</sup> At that time it was believed that alcohol had an acute, and short-lived appetite-stimulating effect, but the underlying mechanism remained unknown.

During the latter half of the 20<sup>th</sup> century hunger-satiety-regulating areas in the brain were discovered.<sup>6,7</sup> It soon became clear that neurons in these areas produced peptides which signaled to other appetite-regulating neurons in the brain.<sup>8</sup> It also became obvious that appetite regulating neurons in the brain received signals from the periphery, giving information about current peripheral energy supplies.<sup>9</sup> These signals were mediated by circulating hormones<sup>10</sup>, and to a great extent conveyed via afferent vagal fibers to neurons located in appetite-regulating centers in the brain.<sup>11</sup>

In modern time several clinical studies have confirmed that alcohol has appetite-stimulating properties in humans.<sup>12,13</sup> However, not all investigators agree on that.<sup>14,15</sup> Moreover, it still remains to be shown whether or not alcohol affects the secretion of newly discovered hormones, reported to be involved in appetite regulation.

This project was initiated with the intention to study if, and how, acute ingestion of alcohol influences some peripherally produced factors/hormones which appear to be of importance for human appetite regulation.

## **5 ALCOHOL AND ENERGY INTAKE**

Alcohol is a potent macronutrient, rich in energy, and yielding 7 kcal/g (29 kJ/g) when metabolized. This means that 10 cl of whisky gives an energy provision of 266 kcal.

Only fat amongst the macronutrients are more potent as an energy source. Among the macronutrients a satiety hierarchy is suggested in a sequence from the most satiating protein > carbohydrates > fat,<sup>16,17</sup> while alcohol is ranked as the forth least satiating of the macronutrients in healthy subjects.<sup>18</sup>

Alcohol is mainly metabolized along two pathways.<sup>19</sup> One of these uses alcoholdehydrogenase (ADH) to transfer hydrogen from alcohol to nicotinamide adenine dinucleotide (NAD), thereby forming NADH. Acetaldehyde is produced by this oxidation. This is further oxidized to acetate and then finally to acetyl-CoA which enters the citric acid cycle. Every mole alcohol being metabolized along this pathway yields 16 moles of adenosine triphosphate (ATP). In individuals with high and chronic alcohol consumption, ethanol is mainly oxidized and degraded by the hepatic microsomal ethanol oxidizing system (MEOS).<sup>20</sup>

It has been a matter of debate whether or not alcohol, given shortly before, or together with a meal, affects the size of the meal.<sup>21-23</sup> Increased energy intake when a meal is taken together with alcohol has been found to be 20 – 30 %.<sup>12,24</sup> In other studies alcohol has had little effect on the meal size,<sup>15</sup> or even caused a decrease in meal size.<sup>14</sup>

The complexity of how alcohol and expectancy on a meal with alcohol affects food intake is well illustrated by two studies carried out by Yeomans et al. In the first of these investigations participants were given carbonated fruit juice to mask the alcohol taste, and rated a reduction in appetite both with this drink and with control drinks. The participants – who did not know whether they had been given alcohol-containing fruit drink, or just carbonated juice before food – were then studied on a second occasion.

On that occasion no alcohol had been added to the juice. They were asked to rate changes in appetite after these two pretreatments, and it turned out that the alcohol containing drink had appetite-stimulating properties compared with the control drink.<sup>24</sup>

In the second study participants knew when alcohol was served. In that setting appetite rating was not similar following alcohol or control, but spirits delayed the appearance of satiety, resulting in a greater energy intake after alcohol than after placebo.<sup>25</sup>

If regular intake of alcohol, over long periods of time, influences the energy balance, is also controversial. Colditz et al found a positive relation between food intake and alcohol consumption in both men and women.<sup>26</sup> However this does not necessarily mean that the body weight increases during long-term alcohol ingestion.<sup>22,27</sup> BMI in men - and increased waist hip ratio in both sexes has been found to be positively correlated to regular moderate alcohol intake.<sup>28</sup> Others have not been able to confirm a positive relationship between BMI and alcohol, and have therefore maintained that alcohol may not have long standing effect on human body weight.<sup>29</sup> In epidemiological studies regular intake has been reported to increase,<sup>30</sup> or lack influence on<sup>31</sup> or even decrease body weight.<sup>32</sup> These rather disparate findings cannot be explained easily, but failure to adjust for smoking, is one possibility as smoking is associated with a decreased energy intake.<sup>33</sup> Failure to take sex into consideration is another possibility Men with a regular alcohol intake are especially liable to weight gain, and even more so obese men.<sup>29</sup> The reason for this is not fully understood but could be due to the fact that men add energy, contained in alcoholic drinks, to the energy content in their ordinary food, whereas in women alcohol energy appears to displace other energy sources.<sup>34</sup> Still, the mechanism how alcohol affects energy intake is unclear. It is known that alcohol has a capacity to suppress fatty acid oxidation, especially in the liver.<sup>35</sup> Suppression of fatty oxidation increase food intake in humans.<sup>36</sup> Some investigators support the idea that energy expenditure is raised following intake of alcohol,<sup>37</sup> while

others report no difference.<sup>15,38</sup> It cannot be excluded that alcohol-induced energy expenditure differs between the sexes. It also possible that peripherally produced hormones known to influence central appetite regulation, are affected differently in men and women. However, nothing is known about that, and it might even be questioned whether alcohol influences such hormones at all. To investigate the effect of alcohol on these hormones has been the objective of this research project.

## 6 APPETITE REGULATION

Obesity is an increasing pandemic, and frequently causes disease and mortality in the western world.<sup>39</sup> As energy rich food now is easily available and a more sedentary life style is common, two driving forces continue to further increase obesity. During the last decade researchers have gained increasing knowledge about mechanisms that regulate appetite and weight control.

The appetite is regulated by a complex system of biologic and cultural factors.<sup>40</sup> It has been known for long that damage to centers within the hypothalamus causes hyperphagia if inflicted in the dorsomedial paraventricular nucleus,<sup>41,42</sup> whereas on the contrary damages in lateral hypothalamus leads to decreased food ingestion.<sup>7</sup>

In rat experiments several peptides have been found acting within these areas in the hypothalamus. This part of the brain receives both neural and hormonal influence, not only from other areas of the brain, but also from the periphery. An early attempt to explain plausible mechanisms underlying hunger and satiety focused on glucose. It was maintained that hypothalamic nuclei were able to monitor changes in blood glucose concentration, and induce increased food intake during hypoglycemia, and decreased intake during hyperglycemia. This hypothesis was called the glucostat theory.<sup>43</sup> It turned out to be much too simple, and was soon abandoned. Another hypothesis was based on energy rich molecules as ATP and changes in body temperature<sup>44</sup>. Hunger would be a consequence of low ATP production and reduced body temperature.<sup>45</sup> Also this hypothesis had to be discarded as it could not be applied on normal-life situations. Today, hunger – in response to low body temperature or hypoglycemia - is considered to be an emergency response.

A third model for regulation of energy balance is the lipostat theory. In this model peripheral signals communicate information about the energy status to brain centers

regulating food intake within the hypothalamus and the brain stem.<sup>9,46</sup> The hypothalamic neurons produce neuropeptide Y (NPY) and pro-opiomelanocortin (POMC) which in humans brings about up- and down regulation of energy intake, respectively.<sup>47-49</sup>

In mice, an increasing number of other centrally produced peptides have been identified, which appear to be involved in the hunger-satiety regulation.<sup>50</sup> However, their role in human appetite regulation is not yet fully elucidated.

The current concept is that circulating factors act together with neural processes to relay and integrate information on the need for fuels and the levels of stored energy for long-term use.<sup>47,51,52</sup> This concept has recently been reviewed.<sup>53</sup> In the hypothalamus five peptides stimulating feeding stand out; NPY, agouti-related peptide (AgRP), melanin-concentrating hormone (MCH), galanin and the orexin. A number of hypothalamic peptides are known to suppress feeding; pro-opiomelanocortin (POMC), galanin-like-peptide, cocaine and amphetamine-regulated transcript (CART) and corticotropin releasing factor (CRF).<sup>47,50,54</sup> In the periphery, as a contrast, only one hormone is considered as orexigenic. This hormone is ghrelin.<sup>55</sup> Several peripherally produced satiating hormones are known i.e cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), leptin, insulin and peptide YY (PYY).<sup>53,56</sup> Small amounts of some of these peripheral hormones are also produced and secreted in the brain and in the hypothalamus.<sup>50</sup> Whether small changes in their central secretion can be detected by hormone analysis on blood, drawn from peripheral sites, is currently unknown.

Most research has been focused on NPY and POMC. These systems can counteract one another. NPY/AgRP neurons are orexigenic, and are co-expressed in the medial part of the hypothalamic arcuate nucleus (ARC).<sup>57,58</sup> whereas the anorexigenic molecules POMC and CART are expressed in the lateral arcuate.<sup>59</sup> (Fig. 1). The orexigenic effect of ghrelin is mediated by NPY/AgRP neurons.<sup>60</sup> Recent studies

suggest that ghrelin induces changes in mitochondrial respiration, which is necessary for mitochondrial proliferation and electric activation of NPY/AgRP neurons.<sup>61</sup> Leptin receptors are found on both NPY/AgRP and on POMC neurons. Both types of neurons are, in an opposing manner, regulated by leptin as this hormone inhibits NPY and stimulates POMC, and both these actions limit energy intake.<sup>62</sup> Ghrelin antagonizes leptin regulation of NPY/AgRP as these neurons are stimulated by ghrelin.<sup>63</sup> An imbalance in this system is suggested to decrease hunger in the ageing where pre-prandial leptin levels were increased and did not change as a response to a meal, while ghrelin levels decreased post-prandially as in a younger control population.<sup>64</sup>

Woods divides peripheral signals into three categories.<sup>56</sup> *Satiety signals* released from the gastrointestinal tract during meals induce satiety - brings a meal to an end, and are reflected by increased secretion of CCK. *Adiposity signals* which are produced in proportion to adipose stores - are reflected by increased peripheral levels of leptin and insulin. Both of them reduce food intake by stimulating POMC neurons in the brain. Adiponectin represents a different type of adiposity signal. The hormone is produced by fat cells but in a proportion inversely proportional to body fat.<sup>65</sup> Low levels of adiponectin are seen in metabolic syndrome, as the release of this hormone decreases in parallel with an increase in visceral fat cell mass.<sup>66</sup> *Neural circuits* within the brain integrate these peripheral signals with neurotransmitters from within the brain, and induce catabolic and anabolic responses.<sup>56</sup>

A number of peripheral factors, which induces satiety, increase after food intake. Such factors are insulin, PYY, GLP-1, CCK and gastrin releasing peptide (GRP).<sup>67</sup> Most of them decrease gastrointestinal motility and exert hypothalamic modulator properties as well.<sup>68</sup>

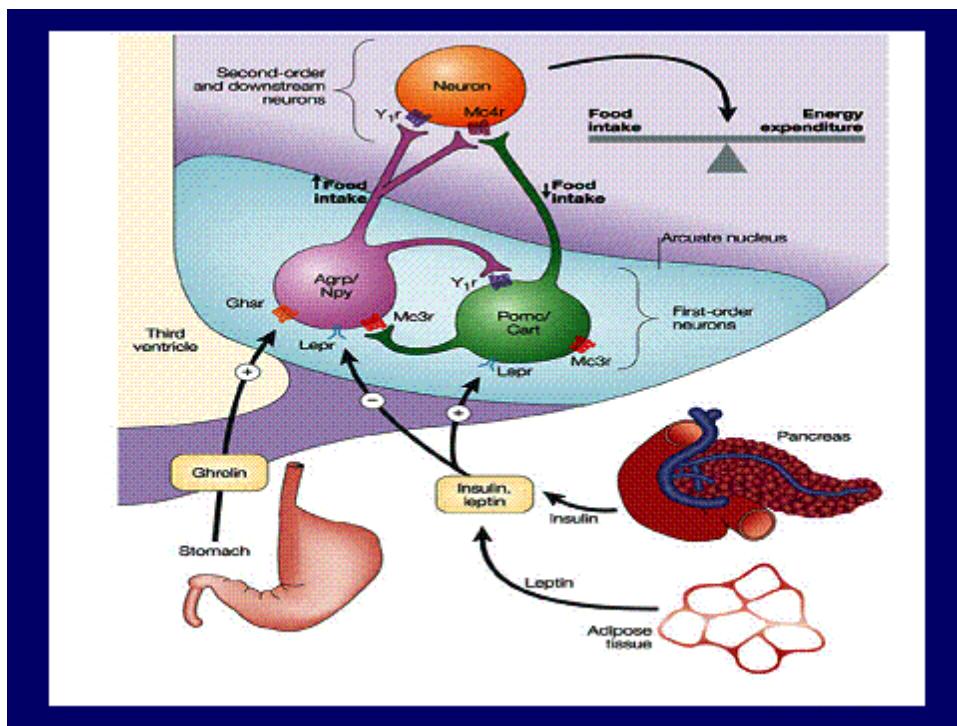


Fig 1. Description of neurons in the arcuate nucleus within the hypothalamus receiving influence from the periphery and controlling energy homeostasis, Gregory S. Barsh & Michael W. Schwartz *Nature Reviews Genetics* 3, 589-600 (2002).  
Permission granted from the publisher.

Hypothalamic centres adjust to nutrient availability as signals within the CNS are integrated with peripheral signals, giving information about actual energy stores and needs.<sup>54,69</sup>

However, recent evidence suggests that hypothalamic neurons - by unknown mechanisms - also are capable of sensing circulating nutrients directly.<sup>70</sup> Moreover, cross talk between the brain and the periphery can go in both directions. In mice, oleic acid injected centrally inhibits food intake and decreases hepatic glucose production.<sup>71</sup> A direct effect of melanocortin system on peripheral lipid metabolism independent of

nutrient intake has also been described.<sup>72</sup> In that study stimulation of the central melanocortin receptors induced peripheral lipid mobilisation, whereas inhibition of these receptors caused increased lipid uptake and fat storage.

Cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6) are also of interest in this context, as they can have inhibitory influence on food intake.<sup>73</sup> Possibly these inflammatory mediators are important in dysregulating food ingestion in disease as cancer, chronic inflammation and end stage renal disease.<sup>74</sup> Administration of IL-1, IL-6 and TNF- $\alpha$  reduce food intake.<sup>75</sup> Peripherally produced cytokines signal to the brain via afferents running in the vagal nerve. Cytokines, produced centrally, may have direct effect on appetite-regulating neurons in the brain, as evidenced by the fact that IL-6 receptors have been found in the NTS, ARC and in the PVN.<sup>76</sup> Cytokines have also been found to modulate gastric motility and emptying,<sup>76</sup> and are able to induce the release of peripheral hormones as insulin, leptin<sup>76</sup> and glucocorticoids,<sup>75</sup> which are known regulators of appetite and hunger.<sup>77</sup> It is interesting to note that in cell cultures of human monocytes, cytokines as IL-6, IFN $\alpha$  and IFN $\gamma$  are found to decrease after acute exposure to alcohol.<sup>78</sup> This finding lends support to the notion that acute ingestion of alcohol may stimulate appetite in humans.

## **7 PERIPHERAL HORMONES**

### **7.1 GHRELIN**

When discovered 1999 ghrelin was at first recognized as the natural ligand to the orphan growth hormone secretagogue receptor.<sup>55</sup> Previous studies, investigating synthetic growth hormone secretagogues had previously characterized this G-protein-coupled receptor.<sup>79</sup> As ghrelin was proved to have appetite stimulating properties, most research concerning ghrelin has been directed to this aspect.

Ghrelin appears in two forms: total ghrelin, which is not known to possess any endocrine action in humans, and octanoylated ghrelin which exerts endocrine effects.<sup>80</sup> The latter form differs from total ghrelin by a *n*-octanoic acid being attached at the third serine residue of the molecule. By octanoylation ghrelin becomes acylated, in contrast to total ghrelin, which is non-acylated. Total ghrelin is more abundant, and it has a 4 hour half life, compared to 8 minutes for its active form. It is not yet known how acylation is brought about, or which enzymes that are responsible for the acylation.<sup>80</sup>

Ghrelin synthesis is regulated by nutritional status. The level of circulating ghrelin is proportional to the amount of calories ingested, and fluctuates diurnally in relation to meal intake.<sup>81</sup> Blood levels are found to be high in lean people and low in obese, age matched subjects.<sup>82,83</sup> After acute caloric intake levels decrease.<sup>84</sup> They increase in fasting,<sup>81,83</sup> and in starvation and anorexia nervosa.<sup>84</sup>

Ghrelin acts as a meal-initiator as it increases rapidly before eating commences and declines within minutes when food is ingested.<sup>81,86</sup> These hormonal changes could be related to daily routines and anticipatory effects could also be of importance.<sup>86</sup>

Ghrelin is mainly produced in the stomach and to a much lesser degree also in the bowel, pancreas, kidney, testes, pituitary, lungs and hypothalamus.<sup>87,88</sup> Using real-time PCR small amounts of ghrelin has also been found in the myocardium, in adipocytes,

ovaries, prostate, liver and adrenal glands.<sup>89</sup> Pre-pro-ghrelin is a 117-residue hormone, which by post-translational cleavage becomes ghrelin- a 28-amino acid peptide.

Ghrelin is synthesised by X/A-like cells in the fundus region.<sup>87,90</sup> (Fig 2). These ghrelin containing entero-endocrine cells do not have contact with the lumen, and are thought to respond to physical or chemical stimulation. Such stimulation release ghrelin to the surrounding capillary network to exert classical endocrine functions.<sup>87</sup> Whether nutrient sensing is mediated by the ghrelin producing cells themselves or by hormonal or neural intermediate signals is not known.

Ghrelin stimulates hunger via effect on neurons in the hypothalamus. This hormonal signal is thought to be relayed by afferent vagal neurons, as the ghrelin increase in fasting rats is abolished by vagotomy,<sup>91</sup> and ghrelin passes only minutely through the blood-brain barrier.<sup>92</sup> Atropine - a cholinergic antagonist which blocks efferent vagal signals - also inhibits ghrelin increase in food deprived rats.<sup>91</sup> indicating that efferent vagal signals also mediate the effect of ghrelin. Intravenous infusion of ghrelin in healthy subjects increases gastric emptying rate in healthy subjects but does not add to the appetite stimulating effect of the hormone.<sup>93</sup>

In the brain ghrelin is found in the ARC in the hypothalamus. In this area ghrelin containing neurons send efferent fibres to NPY and AgRP and might this way stimulate these orexigenic peptides.

Based on rodent experiments ghrelin is a growth hormone (GH) releasing agent.<sup>94</sup> From hypothalamus projections lead to the pituitary where ghrelin stimulate GH release, acting on the growth hormone secretagogue receptor. GH can also be released through direct stimulation on cultured pituitary cells.<sup>55</sup> The hypothalamic stimulation of GH release secondary to ghrelin stimulation has however been questioned. Other factors are thought to be necessary as a complement to ascertain this effect. These factors could be growth hormone releasing hormone (GHRH) as co-administration with this substance

and ghrelin synergistically increases GH secretion.<sup>95</sup> This other factor could be a function of the vagus nerve, as GH release after ghrelin injections following vagotomy are decreased.<sup>87,91</sup> Secretion is not significantly related to concentrations of circulating GH or its mediator insulin-like growth factor-1 (IGF-1).

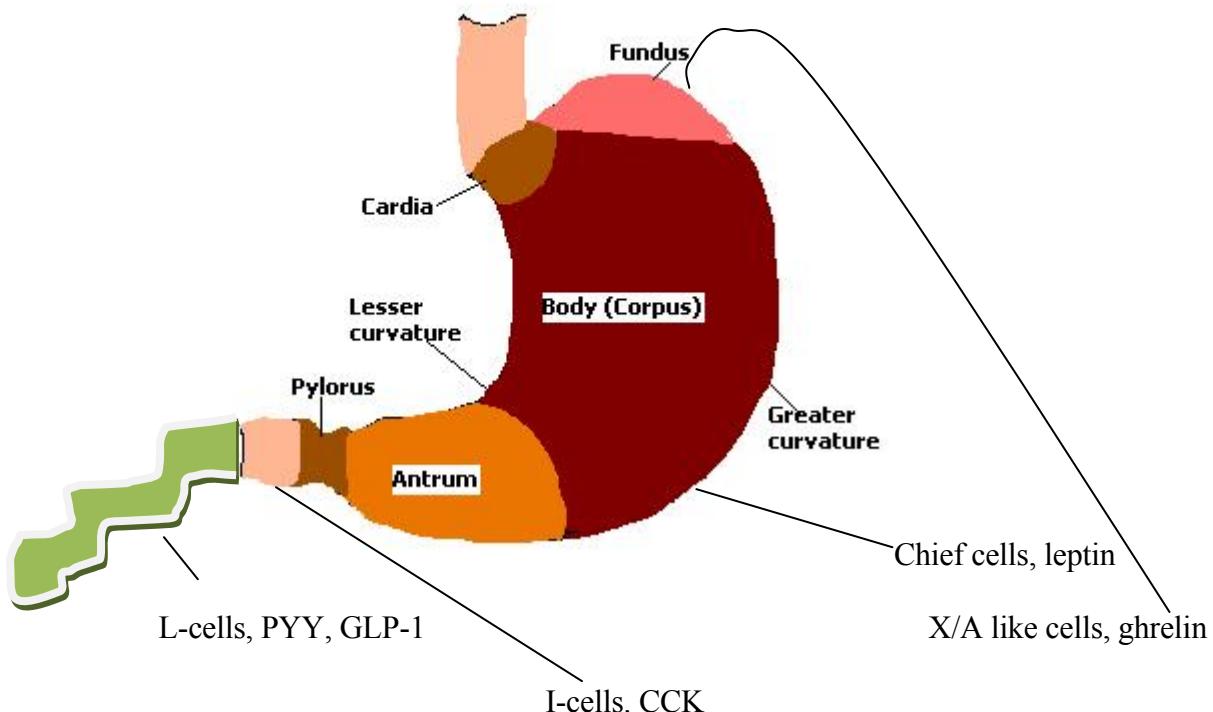


Fig2. Stomach, its parts and cellular origin of appetite regulating hormones  
Modified from <http://www.histopathology-india.net/Stomach.htm>

## 7:2 LEPTIN

The main source of leptin is the adipose tissue. Small quantities are also secreted by the placenta, by skeletal muscle cells, and by chief cells in the distal part of the stomach.<sup>96</sup> Leptin is a 16 k Dalton peptide synthesized by adipocytes. It plays an important role in regulation of food intake and energy balance.<sup>97</sup> Leptin functions like a brake on NPY. Low leptin levels in serum relax this brake, which results in an increase of NPY concentration and augmented appetite. It has also weight regulating-properties caused by increased thermogenesis.<sup>98</sup>

The weight regulating properties were discovered in rodents lacking leptin in the *ob/ob* mouse, or expressing impaired leptin receptors in *db/db* mouse, both manifested in leptin deficiency which resulted in obesity, infertility and early onset diabetes.<sup>99</sup> Studies in humans point in the same direction, as recombinant leptin therapy has proved successful as a weight controlling tool in two young siblings with congenital leptin deficiency, and with an extreme obese phenotype, which was abolished by leptin substitution.<sup>100</sup>

In the normal, non-obese population serum levels are higher in women than in men, reflecting an increased fat mass in women.<sup>101</sup> Obese men and women, in contrast to the *ob/ob* mouse, has higher leptin levels, than their non-obese counterparts. During fasting leptin levels are low, and circulating leptin increase following food intake.<sup>102</sup> Leptin levels are increased by insulin<sup>103</sup> and cortisol<sup>104</sup> and decreased by androgens<sup>105</sup> and catecholamines.<sup>106</sup> In patients with chronic renal disease IGF-1 also appears to down-regulate leptin.<sup>107</sup>

In obese subjects leptin traverses the blood-brain barrier with difficulty,<sup>108</sup> and it has diminished effect at its hypothalamic target. This results in a relative leptin resistance in such individuals. Centrally, leptin and insulin acts in concert. Both peptides stimulate anorexigenic POMC and inhibit NPY, which brings about satiety. The kidneys remove leptin from the circulation, and as a rule leptin is undetectable in urine.<sup>109</sup>

How acute ingestion of alcohol influences circulating leptin in normal individuals had not been given much attention by the time this investigation started.

### 7.3 PEPTIDE YY

Peptide tyrosine tyrosine, PYY, was discovered in the early 80-ies.<sup>110,111</sup> This peptide is a 36-amino acid gastrointestinal hormone synthesized by L-cells in the intestinal mucosal membrane. L-cells are more frequent in the distal part of the intestine than in

the proximal part and are abundant in the rectum. The peptide has a half life of 8 minutes.<sup>112</sup> It exerts central effects via signals running in the vagus nerve. These signals are relayed via NTS and further to hypothalamic neurons.<sup>68</sup> PYY has been found in the central as well as in the peripheral nervous system.<sup>113</sup> The hormone appears in two forms; PYY<sup>1-36</sup> and PYY<sup>3-36</sup>. The enzyme generating PYY<sup>3-36</sup> is dipeptidyl peptidase IV (DPP-IV).<sup>114</sup> Three different receptors are activated by PYY<sup>1-36</sup> (Y1, Y2 and Y5) whereas PYY<sup>3-36</sup> specifically activates Y2 receptor.<sup>115</sup> Studies in rodents imply that PYY affects feeding not only by activation of Y2 receptors on inhibitory POMC neurons, but also by strong inhibitory influence on NPY neurons in the ARC.<sup>116</sup> PYY is released post-prandially in proportion to the calories ingested.<sup>117</sup> It inhibits feeding in rats<sup>118</sup> and may have similar effect in man.<sup>118</sup> However, Batterham's findings showing a decreased food intake and weight reduction in obese subjects after iv administration of PYY,<sup>119</sup> has been difficult to reproduce by other investigators.<sup>120</sup> Therefore, additional studies are needed to decide whether or not PYY contributes significantly to the regulation of the human caloric homeostasis.

#### 7.4 GLUCAGON-LIKE PEPTIDE-1

Pre-pro-glucagon is synthesised by intestinal L-cells (Fig 2). These cells also harbour PYY, and as reported above (7.3) these cells are more frequent in the distal than in the proximal part of the intestine. GLP-1 and PYY are products of the same precursor protein.<sup>121</sup> By processing the pre-hormone, glucagon-like peptide-1 is synthesised. There are two circulating forms of GLP-1; GLP-1(7-37) and GLP-1(7-36) amide. These two hormones appear to be equipotent. GLP-1(7-36) is more abundant in the circulation after a meal than GLP-1(7-37).<sup>122</sup>

Plasma levels of GLP-1 are low in the fasted state, but increases rapidly after a meal, in proportion to ingested calories.<sup>123</sup> The increased GLP-1 level declines when the L-cells

are no longer stimulated by food, due to renal clearance and DPP-IV inactivation.<sup>124</sup>

GLP-1 has a half life of 17 minutes.<sup>125</sup>

Kreymann et al were the first to observe that the GLP-1 level increased significantly in response to food.<sup>126</sup> They suggested that GLP-1 should be regarded as a physiological incretin in man. Gastrointestinal hormones, showing insulinotropic properties in response to oral carbohydrates, are considered incretins. Gutniak et al were able to confirm that GLP-1 fulfilled the above mentioned criteria. They also reported inhibitory influence of GLP-1 on somatostatin and glucagon production in patients with type 2 diabetes.<sup>127</sup> These findings were of great interest not only to the medical scientists, but also to the pharmaceutical industry. Today in the treatment of type II diabetes, both GLP-1 analogues with extended half-life and DPP-IV inhibitors have been developed.<sup>128</sup>

The biologic effect of GLP-1 is mediated by a receptor. This receptor is dependant of glucose concentration and G-protein coupled. In the pancreatic β-cell receptor activation generates cAMP and calcium accumulation, which triggers insulin release.<sup>129</sup> The G-protein receptor is expressed on pancreatic α and β cells, in peripheral tissues as heart, kidneys, lungs and in the CNS, particularly in areas known to be of importance for hunger-satiety regulation like the NTS and in the hypothalamus.<sup>130</sup> Rapid increase of GLP-1 concentration 10-15 minutes following a meal has been interpreted as a result of neural or humoral signals from the proximal gut to the distal L-cells, rather than a direct response to nutrients from the L-cells themselves.

GLP-1 has anorexogenic properties, as evidenced by findings reported by Naslund et al. They gave GLP-1 sc to obese individuals prior to meals, and reported decreased food intake and weight reduction after such treatment.<sup>131</sup> Decreased gastric motility, and GLP-1 influence on central neurons involved in appetite regulation, may at least in part explain the above mentioned effects of GLP-1 in humans.

## **7.5 OBESTATIN**

Obestatin was recently discovered by Zhang et al. They reported that this hormone was a 23-amino acid peptide, encoded by the ghrelin gene and acting on the orphan G protein-coupled receptor GPR-39.<sup>132</sup> In this first report this peptide was shown to have opposing effects on food intake, body weight and gastric emptying to those of ghrelin. However, both the effect of obestatin contrary to ghrelin, and the assumed effect on GPR-39 have since been challenged.<sup>133,134</sup> Zhang has also failed to reproduce the binding of obestatin to GPR-39.<sup>135</sup> The native receptor to this peptide is thus not yet elucidated. Although the nature of the obestatin receptor still is not known, there is no doubt about the existence of obestatin as a hormone, and it is reasonable to believe that this hormone is involved in hunger-satiety regulation, as circulating levels of obestatin and BMI values are inversely correlated,<sup>136</sup> and hormone levels decline in response to food.<sup>137</sup> Levels of obestatin are higher in females than in males.<sup>136</sup> In rats being injected with obestatin before feeding, decreased antral and duodenal motility was observed after 30-90 min possibly linked to increased CRF activity in the hypothalamic neurons.<sup>138</sup> Most investigations on obestatin has been performed in rodents, in man it is suggested a possible role for obestatin in long-term weight regulation.<sup>139</sup>

## **7.6 INSULIN**

Insulin is synthesized by pancreatic β-cells, and is secreted after a two-step cleaving process of intracellularly stored pre-pro-insulin. Hormone release is induced by elevated circulating glucose and amino acid concentrations, caused by food intake. Insulin regulates plasma glucose homeostasis by stimulation of glucose uptake - primarily in skeletal muscle and in adipose tissue. These effects make insulin an anabolic hormone in the periphery. Insulin also regulates glucose homeostasis by its

capacity to reduce hepatic gluconeogenesis and glycogenolysis. The effect is mediated by hormone binding to its cell surface receptor.<sup>140</sup> This receptor undergoes transphosphorylation reactions, which activate phosphatidylinositol 3-kinase, and subsequent stimulation the glucose transporter GLUT-4 to the cell surface.<sup>141</sup> Insulin also influences lipid metabolism by increasing synthesis of lipids in adipocytes and myocytes.<sup>142</sup> In skeletal muscle tissue GLUT-4 is the rate controlling step for insulin-stimulated glycogen synthesis.<sup>141</sup>

As with leptin, which is abundant in obese subjects, high insulin levels are generally seen in overweight individuals. Peripheral insulin resistance develops secondary to obesity, inactivity and low grade inflammation.<sup>143</sup> As these high levels of endogenous insulin is not always enough to normalize elevated blood glucose variable degrees of manifest diabetes type 2 develops.

Insulin has paradoxical effects on hunger regulating centres. Insulin is not synthesised in the CNS but it passes the blood brain barrier in proportion to prevailing serum insulin levels.<sup>144</sup> Brain tissue is also considered an insulin target,<sup>145</sup> and insulin receptors are widely distributed mainly in the hypothalamus, the olfactory bulb, cortex, cerebellum and hippocampus.<sup>146</sup> Insulin receptors are also highly expressed in the ARC, and appetite and hunger are inhibited by insulin.<sup>147</sup> This effect is accomplished by activation of a membrane-bound tyrosine kinase receptors, which stimulate POMC neurons and inhibits NPY/AgRP neurons.<sup>148</sup>

In most obese subjects both insulin and leptin resistance prevails, and the satiating effect of both hormones are decreased. Leptin resistance is thought to be caused by receptor modification,<sup>149</sup> whereas central insulin resistance – according to Spanswick et al – is due to an inability to activate ATP-sensitive K<sup>+</sup> channels in hypothalamic neurons.<sup>150</sup>

High intake of alcohol is known to result in hypoglycaemia, this is thought to be driven by decreased glycogenolysis and impaired gluconeogenesis.<sup>151</sup> Increased insulin sensitivity may also contribute.<sup>152</sup> It has not been convincingly shown that alcohol has significant influence on insulin secretion in humans, but it has been reported that long-term consumption of moderate alcohol doses reduces the risk of developing type 2 diabetes.<sup>153</sup>

## **7.7 INSULIN-LIKE GROWTH FACTOR-1**

IGF-1 has a molecular weight of 7,5 kDa and a structure resembling proinsulin.<sup>154</sup> Also, the insulin and the IGF-1 receptors are structurally related, as both have two  $\alpha$  and two  $\beta$  subunits. Both insulin and IGF-1 decrease blood sugar in man.<sup>155</sup> The IGF-1 measured in the circulation reflect hepatic production of IGF-1. This production is increased by insulin,<sup>156</sup> GH,<sup>157</sup> and food<sup>158</sup> as adequate supply of energy and protein is needed.<sup>159</sup> IGF-1 is essential for growth and development in man, and of importance for proliferation and differentiation of most cell types.<sup>160</sup> Peak levels are seen at puberty.<sup>161</sup> Thereafter these levels slowly decline. It has been reported that high levels of IGF-1 are correlated to better physical health and psychological well-being.<sup>161</sup> By contrast, low levels are related to impaired cognitive function, and inactivity has a negative effect on IGF-1 secretion.<sup>162</sup> Low IGF-1 production signifies catabolism<sup>158</sup> and is seen in starvation,<sup>161</sup> and secondary to serious disease such as cirrhosis of the liver and cancer.<sup>163</sup> Clinically low levels are a hallmark in children with growth hormone deficiency, and in adults secondary to pituitary disease.

## **7.8 INSULIN LIKE GROWTH FACTOR BINDING PROTEIN-1**

Almost all of IGF-1 (>99 %) is bound to one of six binding proteins (BPs).<sup>164</sup> The most common of these is IGFBP-3, which is regulated by GH and nutritional status. In a

ternary complex of IGF-1, IGFBP-3 and a glycoprotein, the acid labile subunit (ALS) 80-90 % of circulating IGF-1 is bound.<sup>165</sup> This complex has a half-life of  $\approx$  15 h and has a molecular weight of 150 kDa. The concentration of free IGF-1 in the vasculature is regulated by this ternary complex.<sup>166</sup> Free IGF-1 has a very short half life of minutes and the ternary complex is a pool of circulating reservoir of inactive hormone. Only free IGF-1 is considered active at the receptor.<sup>167</sup>

IGFBP-1 is clinically the most important of the BPs. Production of IGFBP-1 is mainly in the liver, and is inhibited at transcription level by insulin,<sup>156</sup> whereas synthesis is stimulated by epinephrin, norepinephrin, glucagon and cytokines.<sup>168-170</sup> Also alcohol appears to stimulate IGFBP-1 production, as circulating levels of IGFBP-1 begin to raise a couple of hours after alcohol ingestion<sup>171</sup> It has a half life of approximately 15 minutes.<sup>172</sup> Serum levels show a diurnal variation, with peak levels at dawn.<sup>173</sup> It modulates the hypoglycaemic effects of unbound IGF-1<sup>174</sup> Although the concentration of binding sites on IGFBP-1 is not sufficient to play a major role in IGF-1 transport, the marked increase in IGFBP-1 concentrations that occurs during fasting implies that it protects the individual from the hypoglycaemic effect of IGF-1 in fasting.<sup>175</sup> Some effects are independent of IGF-1 as IGFBP-1 can stimulate migration of smooth muscle cells,<sup>176</sup> and inhibit of breast cancer cell proliferation.<sup>177</sup>

## 7.9 CORTISOL

Cortisol is a glucocorticoid steroid hormone derived from cholesterol and synthesised in the middle adrenal cortex layer, the zona fasciculata. Both synthesis and secretion is under the influence of pituitary adrenocorticotrophic hormone (ACTH), and cortisol exerts a classic feedback inhibition on the pituitary. The glucocorticoid receptor is unliganded localized within the cytoplasm, but translocates rapidly to the nucleus after hormone binding. The cortisol receptor is a member of a superfamily of ligand

inducible transcription factors which control physiological functions as development, reproduction and metabolism.<sup>178</sup> Cortisol has vast physiological effects. Its anti-inflammatory properties stabilise lysosomes, reduces leukocyte activity and block cytokine production.<sup>179,180</sup> Cortisol also stimulates gluconeogenesis, and induces insulin secretion but also decreases insulin sensitivity.<sup>181</sup>

11- $\beta$  hydroxysteroid dehydrogenases inactivate cortisol reversibly by transforming cortisol to cortisone.<sup>182</sup> By action of hepatic 5 $\alpha$  or  $\beta$  reductases cortisol is rendered water soluble, before it is excreted in the urine or the faeces.<sup>183</sup>

Empirically glucocorticoids are known to stimulate appetite in malignant disease.<sup>184</sup> Experimentally, it has been shown that cortisol stimulate leptin secretion peripherally,<sup>185</sup> but acts as a leptin antagonist centrally, due to NPY stimulation.<sup>186</sup>

## 7.10 TESTOSTERONE

Testosterone is a steroid- hormone synthesized in the testis in men and in the ovaries, adrenal cortex and to some extent in fat depots in women. 27-C-cholesterol is the common origin of these hormones and metabolism is stimulated by pituitary luteinizing hormone and enzymatic degradation in a number of steps to the 19-C-steroid, testosterone. Levels are approximately 10 times more elevated in men, compared to women. The amount of fat located inside the abdominal cavity (intra-abdominal or visceral adipose tissue) is twice as high in men compared to women. Studies have shown that circulating androgens are negatively associated with intra-abdominal fat accumulation in men, which explains an important portion of the link between low androgens and features of the metabolic syndrome, and the differing metabolic profiles and cardiovascular disease risk in men and women.<sup>187</sup> Testosterone exerts anabolic action by both stimulating muscle protein syntheses and decreasing muscle protein degradation.<sup>188</sup> Androgens also stimulate osteoblast but suppress osteoclast function

throughout life, and are necessary for reproductive function. Biological actions are mediated by the androgen receptor (AR), a ligand-dependent transcription factor, belonging to the nuclear receptor superfamily. These AR complexes interact with various factors, co-activators or co-repressors, to modulate transcription of androgen target genes via specific DNA sequences.<sup>189</sup> Testosterone is of importance for leptin secretion, as has been shown in men with prostate cancer who were chemically castrated and developed low leptin levels and also decreased peripheral NPY.<sup>190</sup> In rats testosterone has been implicated to regulate insulin positively<sup>191</sup> and to decrease leptin.<sup>105</sup> If androgens have a function in regulating appetite when alcohol is consumed in healthy man is not known.

## **7.11 PERIPHERAL NEUROPEPTIDE Y**

NPY is a 36-amino acid peptide which is widely spread in the central as well as in the peripheral nervous system, where it often is co-localized with norepinephrine and other neurotransmitters.<sup>192</sup> In the gastrointestinal tract NPY is predominantly localised to intrinsic neurons in the submucous and myenteric plexus which innervate the different layers of the intestinal wall.<sup>193</sup> It belongs to the same family of peptides as PYY, and acts on the same type of transmembraneY receptors.<sup>194</sup> NPY is a powerful vasoconstrictor, and has been found to have antisecretory effects on the mucosa in the upper part of the intestine.<sup>195</sup> It increases in the peripheral blood after a meal.<sup>196</sup> but peripheral NPY has not been implicated as a hormone regulating appetite.

## **7:12 CATECHOLAMINES**

As peripheral hormones adrenaline and noradrenaline are synthesized in the adrenal medulla with adrenaline as the more important hormone, essential in fear and flight

mechanism with stimulating effect on blood pressure, pulse frequency and mobilising energy through lipolysis and promoting cellular uptake of glucose. In CNS noradrenaline acts as a neurotransmitter.

Both in the CNS and in the adrenal medulla the amino acid tyrosine is step wise enzymatically degraded to dopamin, and in a third step to noradrenaline, which in neurons are stored in granular vesicles in postganglionic nerve endings. In the adrenal medulla an additional enzyme, phenyl ethanolamine-n-methyltransferase further converts the major part of noradrenaline to adrenaline. These hormones activate two subsets of receptors;  $\alpha$ 1 and  $\alpha$ 2, and  $\beta$ 1 and  $\beta$ 2, respectively.

Adrenaline is elicited by sympathetic activity; cold, heat, stress, fear and anger, and mobilize energy during alarm situations. Peripheral catecholamines are not thought to have a role in food intake. In the CNS however, noradrenaline and dopamine are vital neurotransmitters with multiple action, and both stimulate and suppress eating depending on adrenoreceptor subtype activation.<sup>197</sup> In humans using amphetamine and nicotine low weight is observed and this has been linked to activation in the brain of these transmitters. Drugs have been developed with weight reducing properties acting on these neurotransmitters. Sibutramine, a serotonin and noradrenaline reuptake inhibitor has been registered for a couple of years.



## **7 AIMS OF THE STUDY**

### **GENERAL AIM**

To study the effect of alcohol on the secretion of some peripheral appetite-regulating hormones in healthy man.

### **SPECIFIC AIMS**

- To investigate – both during the day and night - whether acute ingestion of three moderate doses of alcohol affect serum levels of leptin, insulin, cortisol, testosterone, IGF-1 and IGFBP-1 in individuals of both sexes.
- To find out whether catecholamine production – as reflected by urinary excretion of noradrenaline and noradrenaline – is affected differently by alcohol if the alcohol dose is given against a background treatment with propranolol, as compared with a background treatment with placebo.
- To study whether a single dose of oral alcohol has short term effect on circulating levels of total ghrelin and NPY.
- To investigate if total ghrelin (biologically inactive) and octanoylated ghrelin (biologically active), are affected differently by alcohol; whether the drug has prolonged effect on ghrelin levels, and whether alcohol influences PYY in serum.
- To investigate whether one oral oral dose of alcohol affects serum levels of obestatin and GLP-1, and whether alcohol-induced effects on leptin and ghrelin can be modified by sucralfate gastroprotection.

## **9 MATERIAL AND METHODS**

### **9.1 SUBJECTS**

All together 51 healthy of normal weight subjects were included in the investigation. 26 of them were women, and 25 were men. Their mean age was  $24.3 \pm 0.7$  years and,  $23.3 \pm 0.5$  years, respectively (means  $\pm$  SEM). Women BMIs were  $20.9 \pm 0.3$  and  $23.2 \pm 0.4$  amongst the men. All were free from medication. Neither of the subjects used alcohol other than on social events. They refrained completely from ingesting any form of alcohol during three days prior to each experiment. Each participant has served as his/her own control, which made it possible to evaluate how alcohol and/or other drugs influenced the hormone levels in the blood, as they have been investigated twice, on one occasion they received alcohol, on the other a corresponding amount of water was given. In experiment 2 however, subjects were exposed to alcohol on both test days. In this study either propranolol or placebo was given as an intervention. In study V, which was conducted on four separate occasions, an intervention with sucralfate was made on two occasions, complemented with either alcohol or water. On the other two occasions subjects received only alcohol or water. Subjects have been investigated in the morning (I, II, III, IV), at midday (V), and at night (I). The alcohol gave participants an energy boost of about 270 kcal (III, IV, V) to 660 kcal (I, II) in a 70 kg man and an ethanol concentration within one hour between 12 mmol/l (0.45‰) to 28 (1.05‰) mmol/l in a man weighing as much.

The experiments were performed in research wards either at the Karolinska Hospital (II, III) (Clinical Research Unit) or at Stockholm South Hospital (Metabolic Laboratory) (I, IV, V). During the experiments participants were not allowed to eat. They were resting in a supine position. Venous blood samples were drawn at regular intervals from an antecubital vein which was kept patent during the supine part of the

experiments by a slow drip of normal saline. Each protocol, included in this research project, was approved by ethics committees at the Karolinska Institute in Stockholm.

## **9.2 PROTOCOL**

### **9.2.1 Study 1**

Fourteen subjects participated in this study. They were divided into two groups (I and II). Group I contained 8 individuals (4F/4M) studied during the day (between 08.00 and 14.00 h). Group II contained 6 subjects (3F/3M), investigated during the night (between 18.00 and 8.00 h). In both groups subjects were tested twice. The two tests were performed randomly, one week apart. On one occasion they received three equivalent doses of alcohol (each dose 0.45 g/kg), on the other occasion the ethanol doses were changed to three corresponding doses of water. In group I all subjects were fasted over night. They were then given alcohol/water orally at 08.00, 09.30 and 11.00 hours.

Blood samples were collected at regular intervals before and after alcohol/water ingestion for determination of plasma levels of glucose and serum levels of insulin, leptin, and ethanol. In Group II subjects had an ordinary meal 2 h before the experiments. Alcohol/water was then provided at 18.00, 20.00 and 22.00 hours, and blood samples were drawn during the night for determination, not only of glucose, insulin, leptin and ethanol levels, but also of serum cortisol, testosterone, IGF-1 and IGFBP-1 levels.

### **9.2.2 Study 2**

Seven subjects (4F/3M) were included. Each of them participated in two tests (A and B), which were performed in random order and one week apart. Exp A commenced at 08.00 hours with the participants fasted over night. At that time they were given oral placebo. One hour later one dose of alcohol (0.45 g/kg) was ingested. Two additional

and equivalent alcohol doses were given at 10.30 and 12.00 hours, and a second placebo dose was ingested at 12.00 hours. Pulse rate and serum levels of insulin, IGF-1, leptin, and ethanol were recorded at regular intervals between 08.00 and 15.00. Urinary excretion of adrenaline and noradrenaline (between 08.00 and 15.00 hours) were also determined. In exp B propranolol was substituted for placebo. Forty mg propranolol was given orally at 08.00, and an additional 20 mg was ingested at 12.00. In all other details experiment A and B were identical.

### **9.2.3 Study 3**

Eight participants took part in this study (4F/4M). Each of them took part in two experiments, A and B, which were carried out in random order and one week apart. In exp A all participants came to the research ward after a nights fast and ingested one single dose of alcohol (0.55 g/kg). In exp B the alcohol dose was exchanged to an equal volume of water. Both drinks were ingested at 08.00 hours. Blood samples were drawn 08.00, 08.30 and 09.00 hours in order to measure circulating levels of total ghrelin, cortisol, NPY and ethanol.

### **9.2.4 Study 4**

Twelve subjects were included (6F/6M). Each of them participated in two experiments, A and B, which were conducted in random order, one week apart. Alcohol (0.55 g/kg) was ingested in A, an equivalent dose of drinking water in exp B. Both drinks were ingested at 08.00 after fasting from midnight. Blood samples were collected before and after the drinks at 08.00, 08.30, 09.00, 10.00, 11.00 and 13.00 hours. Serum levels of PYY, total ghrelin, octanoylated ghrelin, and ethanol were determined.

### 9.2.5 Study 5

Ten subjects (5F/5M) were participating. Each of them took part in four experiments WW, SW, WA and SA, which were randomized and carried out 1 – 3 weeks apart. All had an ordinary breakfast at 07.00, 2 hours prior to the start of experiments.

In exp WW an oral dose of drinking water corresponding in volume to the alcohol dose in exp WA was given at 12.30 hours, on a background treatment of two doses of 5 ml water. Those two background doses were given at 09.00 and 12.00 h, as shown in Fig 3.

In exp WA one oral dose of alcohol (0.55 g/kg) was given at 12.30 hours, on a background treatment of water as in exp WW.

In exp SW an oral dose of water was given at 12.30 hours on an oral background treatment with two 5 ml doses of sucralfate, given at 09.00 and 12.00 hours.

In exp SA an oral dose of alcohol (0.55 g/kg) was given at 12.30 hours on a background treatment with sucralfate as in exp SW.

Blood samples were collected at regular intervals before and after the drinks at 09.00, 12.00, 12.30, 13.00, 13.30, 14.30, 15.30 and 16.30 hours. Serum levels of leptin, ghrelin, obestatin and ethanol and plasma levels of GLP-1 were determined.

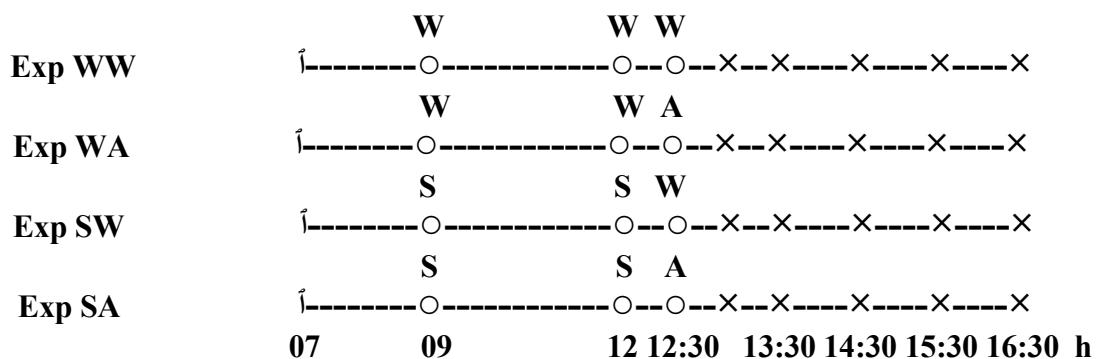


Fig 3. Illustrating test protocol in study 5. W = water, A = alcohol 0,55 g/kg, S = 5 ml sucralfate. Blood samples were collected at X and at times as described above. Subjects fasted after breakfast until final blood samples were drawn 16.30 h.

### **9.3 Serum analysis**

**Leptin:** Serum leptin concentrations were determined in three studies (I, II and V). In the first two of them leptin concentrations were measured with a commercially available radio immunological method with an analysing kit from Linco, St. Charles, USA. The intra- and interassay coefficiencys of variance (CV) were 3,8 – 5.0% and 4.0 – 6.5 %, respectively at leptin serum concentrations ranging between 2.5 - 10 µg/l. The lower detection limit was 0.5µl.

In paper V Leptin was measured with a commercially available RIA-kit from Millipore Corporation, Linco Research, Inc, which was semiautomated and had intra-and interassay CVs of 4.5 and 2.3 %, respectively, at serum concentrations of 3.9 µg/l. The method had a lower detection limit at 0.5µg/L.

**Total ghrelin:** Three somewhat different kits were used in this research project. In study III a radio immunologic (RIA) kit from Linco, St. Charles, USA was used. In this RIA a solubilised calibrator was used. Within respective in between CV was 4.4 % and 16.7 %, at serum concentrations of approximately 3000 pg/ml. In study IV a slightly modified version of the RIA-kit from the same manufacturer was used. It used a freeze dried calibrator, which made calculated serum levels approximately 65 % lower than leptin concentrations recorded in study III. Within CV was 10.0 % and between CV was 14.7%. Limit of detection was 93 pg/ml.

In study V ghrelin was measured with a commercially available RIA-kit from Millipore Corporation, Linco Research, Inc., which was semiautomated and standardized in the

laboratory. A lyophilized ghrelin standard stock at 7500 pg/ml was used, from which dilutions were made. The sensitivity of the method was <100 pg/ml, and the intra- and interassay CVs were 6.4 %, and 2.5 %, respectively at a total ghrelin concentration of 550 pg/ml, and 3.9 and 5.7 %, respectively, at a ghrelin concentration of 1229 pg/ml.

**Octanoylated ghrelin:** Serum concentrations of octanoylated ghrelin was also analysed by RIA-kits from Linco, St. Charles, USA. The intra- and interassay CVs were 6.7 % and 9.6 %, respectively at a ghrelin concentration of 140 pg/ml. The lower detection limit was 7.8 pg/ml.

**NPY:** Plasma concentrations of NPY were extracted with fast face extraction (Sep Pak). P-NPY was measured with RIA- technique. CVs for intra- respective interassays were 7.2 % and 9.3 % at NPY concentrations of approximately 40 pmol/l. The method's detection limit was 1.9 pmol/l.

**PYY':** Serum concentrations of PYY were measured with a RIA-kit from Linco, St. Charles, USA. Within CV was 2.9 % and between CV was 7.1 % at a serum PYY concentration of 111 ng/l. Detection limit was 10 ng/l

**Insulin:** Serum insulin concentrations were determined in study I and II by use of RIA-kits purchased from Pharmacia & Upjohn, Stockholm. Intra- and interassay CVs were 5.8 and 5.8 %, respectively, at an insulin concentration of 11.3.mU/l. The detection limit was < 2 mU/l.

**Cortisol:** Serum concentrations were measured with an immunologic method with an autoDELPHIA instrument, (Wallac Oy, Turku, Finland), using fluorescence detection.

Intra- and interassay CVs was 4.1 and 3.6 %, respectively, at cortisol concentrations of approximately 200 nmol/l. The detection limits was 5 nmol/l.

**Testosterone:** Serum levels of testosterone were determined with RIA (Coat-a-Count, Diagnostics Products Corp., Los Angeles, USA). This method had an intrassay CV of 6 % and an interassay CV of 10 % at testosterone levels of approximately 13 nmol/l.

**Ethanol:** In the first study ethanol gas chromatography with flame ionization detection was used to measure ethanol concentrations in serum Chrompack CP 903 (Varian, Middleburg, Netherlands). In study II- IV measurements were made enzymatically on a Hitachi 911 instrument (Roche Diagnostics, Bromma, Sweden). In study V another enzymatic method was used, Synhron LX (Beckman Coulter, California, USA).

**IGF-1:** Serum levels of IGF-1 were analyzed with a RIA after separation of IGFs from IGFBPs with acid-ethanolextraction and cryoprecipitation.<sup>215</sup> Intra- and interassay CVs were 4 and 11 %, respectively. Detection limit was 8 $\mu$ g/l.

**IGFBP-1:** Serum concentrations were analysed with RIA technique ad modum Póvoa.<sup>198</sup> Intra- and interassay CVs were 3 and 10 %, respectively. The method had a lower detection limit of 2 $\mu$ g/l.

**Glucose:** Plasma glucose measurements are a routine analysis performed by use of a Hitachi 917 analyser from Tokyo, Japan. The analysis is based on a hexokinase method (Boeringer-Mannheim, Germany).

**Catecholamines:** Excretion of catecholamines in urine was measured by high pressure fluid chromatography and electrochemical detection according to Eriksson & Wikström.<sup>199</sup> For adrenaline excretion the intraassay CVs was 7 % at an adrenaline excretion value of 53 nmol/24 h, and 5 % at 173 nmol/24 h. The corresponding noradrenaline CVs at excretion values of 232 and 588 nmol/24 h were 5 and 4 %, respectively. The method had a detection limit for adrenaline at 2.5 nmol/l, and for noradrenaline at 10 nmol/l.

**Obestatin:** Serum concentrations of obestatin were measured with EIA S-1284 (Bachem, Peninsula lab, San Carlos, California, USA). Intra assay CV was < 5, and inter assay CV < 14 %, respectively. The lower detection limit was 0.08 – 1.00 ng/ml. Cross reactivity with ghrelin was zero.

**GLP-1:** GLP-1 concentrations in plasma were measured by RIA after extraction of plasma with 70 % ethanol. Carboxy-terminal GLP-1 immunoreactivity was determined using antiserum 89390 which has an absolute requirement for the intact amidated carboxy-terminus of GLP-1 7-36amide and crossreacts less than 0.01% with carboxy-terminally truncated fragments and 89% with GLP-1 9-36amide, the primary metabolite of dipeptidyl-peptidase IV mediated degradation. The sum of the two components (total GLP-1 concentration) reflects the rate of secretion of the L-cell.<sup>200</sup> Sensitivity was below 1 pmol/l, and intra-assay coefficient of variation below 5 %.



## 10 RESULTS AND DISCUSSION

The objective of the present research project was to study whether alcohol affects peripherally produced hormones known to convey information from the periphery to the brain about the energy balance and energy supplies of the body. If alcohol has such an effect, it would be of interest to know whether it explains the appetite-stimulating effect of the drug many investigators have described previously.

A number of appetite-regulating hormones have appeared in the literature over the last decades. This study has focused on some of them.

Study 1 put emphasis on leptin – a hormone synthesized and secreted by human adipocytes. It inhibits appetite and induces satiety in laboratory animals,<sup>201</sup> as well as in humans with limited effects.<sup>202</sup> In our study 3 small oral doses of alcohol lowered leptin level significantly compared with 3 similar doses of water (**paper 1**). This was an effect which was observed during the day as well as during the night. The underlying mechanism is unknown, but it is likely that alcohol could have influenced fat cells directly, or indirectly, via other hormones or hormone-associated factors. Alcohol inhibition of leptin release from hormone-producing cells in the stomach might also have contributed, since chief cells in the distal part of the stomach appear to be capable of producing small quantities of leptin.<sup>96</sup>

Indirect influence of alcohol on human adipocytes could be exerted by insulin. This hormone stimulates the secretion of leptin.<sup>104</sup> Insulin and leptin have much in common. Both hormones are increased in obese subjects,<sup>47</sup> and both stimulate POMC, and inhibit NPY neurons in the ARC.<sup>56</sup> Consequently, they act in concert to reduce the intake of food. However, in our investigation similar circulating insulin and glucose levels were obtained during the day and during the night, regardless of whether alcohol or drinking

water was consumed (**paper 1**). This makes it hard to believe that insulin is the factor that mediates the alcohol-induced leptin decrease.

Cortisol is known to stimulate leptin secretion peripherally,<sup>185</sup> but acts as a leptin antagonist centrally by stimulating NPY neurons.<sup>186</sup> The NPY stimulation appears to dominate – at least in some situations – as patients with malignant diseases report increased appetite after glucocorticoid treatment.<sup>184</sup> In our study circulating cortisol levels did not differ after ingestion of alcohol as compared to water (**paper 1**), thus suggesting that cortisol is not the factor which mediates the inhibitory effect on leptin secretion.

Testosterone is also of interest in this context, as *in vitro* studies have shown that androgens suppress leptin production in a dose-dependent manner,<sup>105</sup> and hypogonadal men have increased circulating leptin levels, which markedly decrease after treatment with testosterone.<sup>203</sup> If alcohol stimulates the secretion of testosterone a decreased release of leptin would probably ensue, which in turn could have stimulatory influence on appetite. However, also this hypothesis is unlikely, as our study showed similar testosterone levels regardless of whether alcohol or ordinary drinking water was ingested (**paper 1**).

It has been reported that human IGF-1 lowers leptin levels significantly in patients with chronic renal disease.<sup>107</sup> It cannot be excluded that IGF-1 might have similar effect also in healthy individuals. If so, it is tempting to speculate that alcohol – by stimulating IGF-1 production in the liver – inhibits leptin release from peripheral adipocytes, thereby inducing an increased appetite. However, circulating IGF-1 binds to several binding proteins (BPs). One such BP is IGFBP-1. Only unbound IGF-1 is biologically active.<sup>167</sup> This makes it important to investigate how IGFBP-1 influences the secretion of leptin. It has been reported that this BP and leptin are inversely correlated.<sup>204</sup> It has also been shown previously that alcohol ingestion increases the hepatic production of

IGFBP-1, but leaves IGF-1 production practically unaffected.<sup>171</sup> Similar findings were recorded in the present investigation (**paper 1**). This does not favour the idea that alcohol stimulates human appetite by increased hepatic release of IGF-1. IGFBP-1 does not seem to play an important role either, as the leptin level began to fall already a couple of hours before the BP level began to increase (**paper 1**)

If acute ingestion of alcohol stimulates appetite in man catecholamines (CAs) are plausible mediators of this effect, as CAs have been found to inhibit the secretion of leptin.<sup>106</sup> Study II was undertaken with the intention to find out whether alcohol inhibits the leptin secretion indirectly, either via increased CA secretion, or via changed responsiveness to the beta-adrenoreceptor. For that purpose three small oral doses of alcohol were given to healthy individuals against background treatments of either placebo, or a beta-adrenoreceptor blocking agent (propranolol). When alcohol was ingested against a background of placebo the pulse rate increased markedly. This could reflect an increased acetaldehyde production after alcohol ingestion as part of the flush syndrome. However, none of the participants experienced flush and discomfort, thus making this possibility unlikely. Some previous investigators<sup>205,206</sup> – but not all<sup>207,208</sup> have described increased CA secretion/excretion after alcohol. In the present investigation similar CA excretion in the urine was found regardless of background treatment (**paper II**). This makes it reasonable to assume that an increased pulse rate after alcohol may not be caused by increased CA secretion, but could well be a consequence of changed adrenoreceptor responsiveness to CA. The findings also suggest that CA may not be the underlying factor to an alcohol-induced appetite increase.

Many endocrine cells in the gastrointestinal tract are involved in the regulation of food intake. Ghrelin is one of them. It is synthesized and secreted by X/A cells in the stomach, and to a lesser degree also by cells outside the gastrointestinal tract.<sup>87</sup> Ghrelin-

producing cells in the stomach are deeply embedded in the mucosa, and do not have direct access to the lumen. Whether they respond to nutrients indirectly via intermediate hormonal or neural signals, is currently unknown. It is known, however, that ghrelin stimulates food intake, and stimulates gastrointestinal motility causing enhanced gastric emptying.<sup>80</sup>

NPY is also of interest in this context. Besides being active centrally – where it is produced by hypothalamic neurons involved in the hunger-satiety regulation – it is also common peripherally, where it is found in the peripheral nervous system, often co-localized with norepinephrine and other peptide neurotransmitters.<sup>192</sup> In the gastrointestinal tract NPY is predominantly localized in the intrinsic neurons in the submucos and myenteric nerve plexus, innervating the mucosa, submucosa and smooth muscle of the intestinal wall.<sup>193</sup>

After alcohol ingestion the drug is rapidly taken up by the mucosa, traverses the gastrointestinal wall, and reaches the capillaries. During this transit ghrelin-producing X/A cells may get in contact with the absorbed alcohol. Whether ingestion of a moderate amount of alcohol influences the function of these cells was not known at the time of our study. How alcohol affects peripherally produced NPY was also unknown. That was investigated in study III.

Alcohol was given to healthy volunteers on two occasions – on one, they received alcohol orally, on the other, drinking water. The acute ghrelin and NPY responses were determined and compared in the two settings. After alcohol the serum ghrelin level fell rapidly and profoundly as the serum ethanol concentration rose. Water did not affect the ghrelin level at all. The plasma level of NPY remained unchanged by both alcohol and water (**paper III**). As mentioned above ghrelin is known to induce hunger by stimulating NPY neurons in the hypothalamus. A ghrelin decline and an unchanged NPY level peripherally – as were recorded in the present investigation – should favour

satiety rather than increased appetite, and thus contradict the hypothesis that an alcoholic drink, offered before a meal, may serve as an “appetizer”. However, that may not necessarily be the case, as alcohol may have different effect on centrally produced NPY compared with the effect on peripheral NPY. Furthermore, ghrelin appears in two forms – one being biologic active (octanoylated ghrelin), the other inactive (total ghrelin). In study III inactive ghrelin was determined. It is possible that alcohol might have affected total ghrelin differently than octanoylated ghrelin. Study IV was set up to shed light on that issue. The experimental time was extended considerably from 1 hour in study III to 5 hours in study IV. Both total and octanoylated ghrelin were analyzed, and also serum levels of PYY. PYY was considered of interest, as that hormone inhibits feeding in rats by inhibiting hypothalamic NPY, and stimulating POMC neurons in the same area.<sup>116</sup> PYY appears to have similar effects also in humans. It is produced by intestinal L-cells, which increase in number distally, and are most common in the rectal area.<sup>111</sup> In our investigation alcohol failed to affect the PYY-producing L-cells significantly (**paper IV**), but exerted powerful influence on the X/A cells. Both total and octanoylated ghrelin levels fell rapidly (within 30 minutes), and markedly after ingestion of the drug (- 36 and - 48 %, respectively), and remained significantly decreased for a prolonged period of time (> 5 hours). Although these results do not support involvement of PYY or ghrelin in alcohol-induced appetite stimulation, they are of interest from another point of view.

It is well known that strong alcoholic drinks may damage the delicate gastric mucosa and cause ulceration. Under such conditions ghrelin may offer gastroprotection, as shown by a study in mice. When ghrelin was administered to animals which were also given alcohol, they developed fewer and less extensive ulcers than those given alcohol alone.<sup>209</sup> If so, our current results – demonstrating decreased ghrelin secretion after alcohol ingestion- could at least in part explain why alcohol often has deleterious

influence on the human gastric mucosa. Moreover, leptin has also been suggested to be a player in the mucosal defence, thus a decreased leptin secretion could further exacerbate the risk of ethanol damage.<sup>210</sup>

Study V had two purposes. One was to find out whether a gastroprotective agent – sucralfate - would modulate the alcohol-induced responses, which we found in our previous studies. Another purpose was to explore additional gastrointestinal hormones with appetite-regulating properties, in order to see whether they would mediate the appetite-stimulating effect of the drug. Two hormones were studied: GLP-1 and obestatin. GLP-1 decreases food intake by inhibiting hunger-stimulating neurons in the hypothalamus,<sup>130</sup> and by decreasing gastric motility.<sup>68</sup> Obestatin acts similarly, and has an inhibitory effect on food intake, body weight and gastric emptying, opposite to those of ghrelin.<sup>132</sup> In our study neither of these two hormones were affected by the alcohol ingestion (**paper V**).

Sucralfate is a pharmacological agent which has been used in clinical praxis to heal gastric ulcers, and to protect gastric mucosa from damaging influence of compounds like aspirin and ethanol. When our participants were ingesting alcohol against background treatments of sucralfate or water, sucralfate proved to have no significant influence on the alcohol-induced hormone responses (**paper V**). This could either mean that the dose of sucralfate was too small to offer adequate gastroprotection, or that alcohol affects the secretory cells further down the intestine than can be reached by oral sucralfate. It is not very likely that cells producing leptin are found in the distal part of the intestine. If so, they would probably contribute only insignificantly to the total amount of circulating leptin, most of which is released from peripheral fat depots.

Accordingly, it should be rather difficult to detect small changes in the secretory pattern of these gastric leptin- synthesizing cells after alcohol ingestion, provided that the measurements had been made in blood from a peripheral vein. The opposite applies to

ghrelin. The major proportion of ghrelin, appearing in the circulation, originates from secretory cells in the gastrointestinal wall.<sup>55</sup> Consequently, small changes in their secretory pattern should be easier to discern in blood from a peripheral vein. The falling level after alcohol is maybe less important in this context, as we were interested in finding a link between appetite and peripheral hormones. A ghrelin-decline has an appetite-lowering effect. A leptin decline, on the other hand, has appetite stimulating effects, and could hence be the link we are looking for. However, also this may not be correct, as we measured acute leptin changes in response to alcohol, and leptin is known for long-term, rather than short-term effects on appetite regulation.

This research project has been able to demonstrate influence of alcohol on peripherally produced hormones, but has failed to present a relationship between hormonal changes and a presumed appetite increase after alcohol. There is no obvious explanation to this, but it cannot be excluded that alcohol stimulates appetite mainly via central influences. If the effect is exerted directly on inhibitory (POMC), or stimulatory neurons (NPY), is unknown, and difficult to determine in living humans, but some investigators have shown that rats – after prolonged exposure to ethanol (12 weeks) – display elevated levels of NPY in neurons found in the ARC,<sup>211</sup> whereas others have reported increased NPY production in central neurons during alcohol withdrawal.<sup>212,213</sup> It may be objected that the alcohol doses might have been too small to affect some of the hormones tested in this research project, and that they – as a consequence – also have been too small to influence the appetite. However, that is probably not the case for the following reasons: 1) The alcohol doses did in fact influence the secretion of both leptin and ghrelin. 2) Caton et al gave their participants either no alcohol, 8 g or 32 g of the drug 30 minutes before lunch, and found a significant increase in both hunger ratings and food intake, after ingestion of the high dose, but not after the low.<sup>214</sup> They speculate that there might be a threshold for alcohol to stimulate hunger. In our study

all doses of alcohol were well above 32 g (33 – 115 g for subjects ranging in weight between 60 – 85 kg).

	<b>Effect of alcohol</b>	<b>Ref</b>	<b>Year</b>
Leptin	↓	Röjdmark	2001,
	↓	Calissendorff	2004, submitted
Cortisol	-	Waltman	1993
	-	Röjdmark	2001
Insulin	-	Trojan	1999
	-	Röjdmark	2001
	↑ (in alcoholics)	Adinoff	2003
Testosterone	↓ (1.5 g/kg)	Heikkonen	1996
	-	Röjdmark	2001
IGF-1	-	Röjdmark	2001,
	-	Calissendorff	2004
	-	Grønbæk	2005
Free IGF-1	↓	Grønbæk	2005
IGFBP-1	↑	Knip	1995,
	↑	Röjdmark	2000, 2001
GLP-1	-	Svartberg	1998
	-	Raben	2003
	↓ (ingested with fat)	Daalgard	2004
	-	Calissendorff	submitted
Total ghrelin	↓	Calissendorff	2005, 2006
	↓	Zimmermann	2007
Octanoylated ghrelin	↓	Calissendorff	2006
PYY	-	Calissendorff	2006
Obestatin	-	Calissendorff	submitted
Catecholamines	-	Trojan	1999
	-	Calissendorff	2004
NPY (peripheral)	-	Calissendorff	2005

Tab 1. Peripheral hormones and factors after moderate alcohol intake, results in this thesis and previous investigations.



## **11 CONCLUSIONS**

If alcohol has appetite-stimulating properties in humans, one would expect to find inhibitory influence of alcohol on leptin secretion, and on IGF-1 bioavailability, since low levels of both these factors inhibit hunger and intake of food. One would also expect increased secretion of ghrelin, as this hormone stimulates hunger. Contrary to that assumption the ghrelin level fell markedly in this investigation. Although the leptin level and IGF-1 bioavailability also decreased significantly, this does not necessarily mean that the alcohol-induced leptin change is the mechanism that underlies alcohol-induced appetite-stimulation. This is because the leptin level began to fall shortly after the alcohol intake, and previous studies have implied that leptin may have long-term rather than acute inhibitory effect on hunger. Therefore, the present findings do not lend support to the notion that alcohol has stimulating effect on the appetite by influencing peripherally produced hormones. It is more likely that an appetite-stimulating effect is caused by direct influence of alcohol on appetite-regulating neurons in the CNS.

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